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INFLUENCE OF PITUITARY GROWTH HORMONE ON GROWTH AND METASTASIS FORMATION OF A TRANSPLANTABLE MOUSE SARCOMA[1]

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The Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts

Received for publication September 15, 1954

The pathological process of tumor metastasis includes the entry of tumor cells into blood or lymph channels, embolization, and lodgment at distant sites with survival and growth there. Though the pathways of spread have been well delineated, the factors involved in the entry of tumor cells into vascular and lymphatic channels and also in the survival and growth at sites of lodgment are less clear. The growth phase of embolic tumor cells is dependent upon the multiplication of tumor cells at their new lodging and upon the development of adequate stroma.

It is well known that the enormous majority of embolic cells fails to become established, for example, in the lung as metastatic tumors (2, 11, 12, 13, 15). Therefore, factors which alter the survival of embolic tumor cells could be of importance in the formation of metastatic tumors.

In the present experiments, it has been found that pretreatment of the host, $C_{57}Bl/6$ mice, with pituitary growth hormone increased the number and size of lung tumors following the intravenous inoculation of tumor cells (sarcoma T241), while treatment with the hormone after tumor inoculation was without effect on the incidence of resulting lung tumors. However, the same amount of growth hormone administered to mice bearing this sarcoma as subcutaneous axillary growths, during the period of spontaneous metastasis to the lung, did not alter either the growth of the primary tumor or the observed number of lung metastases.

Growth hormone was selected as an agent which might affect the local growth and successful establishment of embolic tumor cells, since it was observed that for the transplantable mouse sarcoma employed, larger primary tumors in general were associated with more lung metastases (13), that it metastasized more frequently and caused death more rapidly in the younger animals than in the aged (14), and that it metastasized more rapidly during pregnancy and lactation (14).

[1] This work was carried out under United States Atomic Energy Commission Contract AT(30-1)-901 with the New England Deaconess Hospital.

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[4] Present address: Massachusetts Memorial Hospitals, Boston, Massachusetts.

Smith and associates demonstrated that pituitary growth hormone increased both the body weight and tumor growth rate of C_3H mice bearing a transplanted mammary adenocarcinoma during the first 27 days of tumor growth (9, 10), but that after this stage the effect on the tumor was reversed despite continued increase in somatic tissue (10). Moon and associates (4, 5, 6, 7, 8) have suggested the carcinogenic actions of growth hormone in rats.

MATERIALS AND METHODS

Mice of the $C_{57}Bl/6$ strain obtained from Bar Harbor, Maine, at the age of four to six weeks and maintained on stock Purina lab chow and water *ad libitum* were used throughout the present experiments.

The transplantable tumor used in these investigations is designated as T241[5], a rapidly growing, pleomorphic sarcoma which metastasized early and frequently to the lungs following subcutaneous implantation (13). Tumor cell suspensions were prepared by mincing the solid tumor with scissors and then pressing the resulting tumor mince through a finely meshed tea strainer. The diluent was either ascitic fluid or physiological saline. The resulting suspensions were centrifuged until microscopic examination of a portion of the supernatant failed to reveal cell clumps. The resulting supernatant contained predominantly single cells. Control and experimental animals were injected in the tail vein alternately with a well shaken tumor cell suspension of the supernatant.

In each experiment, at the time of killing all groups were coded and all lung tumor observations made consistently by one investigator (J. S. W.). In this manner, the lung tumor data were obtained without knowledge of the treatment received by the experimental animals until all animals were killed.

Four separate experiments will be reported, wherein the following three pituitary hormone preparations[6] were employed: (1) growth hormone preparation No. 14496, assayed at 1400 rat units per gram, and containing no significant adrenocorticotrophic (ACTH) activity (Experiments 1 and 3); (2) growth hormone preparation No. 170631, assayed at 1839 rat units per gram, and likewise containing no significant ACTH activity (Experiment 2); (3) thyrotrophic hormone (TSH) preparation No. 50P4 (Experiment 4). Each experimental animal received hormone in 0.5 ml. or less 0.02 M NaOH, pH adjusted to 9.0, and the controls received an equal quantity of vehicle.

RESULTS

Experiment 1. Two groups of mice were used, one injected subcutaneously only with vehicle and the other with growth hormone. In Figure 1, the duration in days of the experiment is recorded on the abscissa while the two vertical lines represent respectively the time of intravenous inoculation of an ascitic fluid suspension of sarcoma T241 cells, and the time of killing of the animals. The mice injected with growth hormone received 3 mgm. before tumor inoculation and 21 mgm. after tumor inoculation. The controls received an equivalent volume of the vehicle.

[5] The transplantable tumor T241 was obtained from Dr. D. R. Coman at the University of Pennsylvania, Philadelphia, Pennsylvania.

[6] The hormone preparations were generously supplied by Dr. D. A. McGinty, Parke, Davis and Company, Detroit, Michigan.

EFFECT OF GROWTH HORMONE ON INTRAVENOUS TUMOR

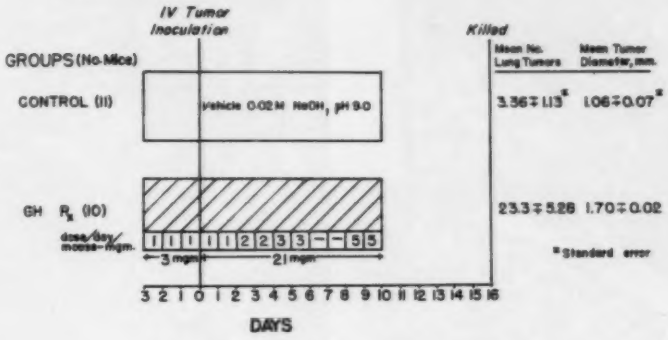

FIG. 1. Experiment 1. For explanation see text.

At autopsy, the lungs were removed and surface tumors greater than 0.2 mm. in diameter were counted with the aid of a dissecting microscope (13). The sizes of the individual tumors were measured with an ocular micrometer. These data are also recorded in Figure 1. The hormone-treated mice showed a six-fold increase in the number of lung tumors, and the tumors in the treated animals were significantly larger. Both of these findings are significant for the number of animals used ($P < 0.001$).

Routine histological preparations were made of the lungs of the control and hormone-treated mice to verify gross counts. Figure 2 shows a typical section of the lungs of a control animal showing tumor.

Figure 3, taken at the same magnification as Figure 2, shows the relatively increased number, size and more extensive growth seen in the growth hormone-treated animals. In the hormone-treated animals an intracardiac tumor was also detected, extending into the myocardium from the right auricle (Fig. 4). More rapid weight gain and hair growth were noted in the growth hormone-treated animals than in the controls.

Methenamine silver staining (3) for stromal reticulum revealed decreased reticular fibers in regions around the lung tumors in the growth hormone-treated animals, as compared with the same regions in the controls. Ultraviolet microscopy and histochemical staining (pyronine-methyl green) by methods described elsewhere (1) showed increased nucleic acids and nucleoproteins in the stromal cytoplasm surrounding the tumors in the growth hormone-treated animals, compared with the controls.

Experiment 2. The objective of this experiment was to determine whether the effect of hormonal therapy was primarily upon the tumor or the host environment. Mice were divided into four groups (Fig. 5): (1) *control group*; (2)

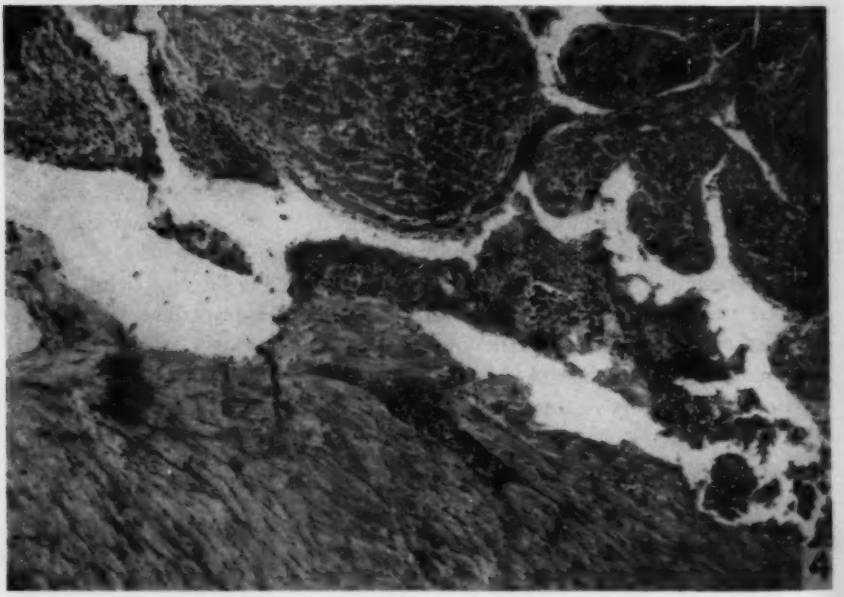

FIG. 2. Metastatic sarcoma T241 in the lung of a control mouse. All sections stained with hematoxylin and eosin. ×25.

FIG. 3. Lung of a growth hormone treated mouse, with more numerous and diffuse T241 metastasis. ×25.

FIG. 4. Subendocardial T241 metastasis in the heart of a growth hormone treated mouse. ×100.

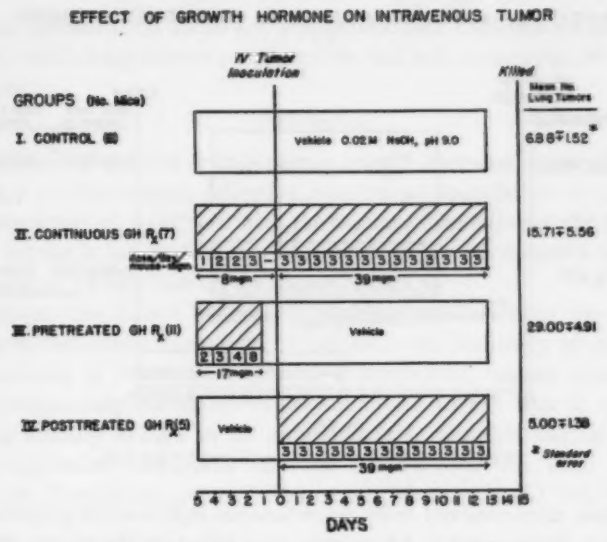
EFFECT OF GROWTH HORMONE ON INTRAVENOUS TUMOR
IV Tumor Inoculation
Killed
GROUPS (No. Mice)
I. CONTROL (60)
Vehicle 0.02 M NaOH, pH 9.0
6.8 ∓ 1.52*
II. CONTINUOUS GH Rx (7)
1 2 2 3 – 3 3 3 3 3 3 3 3 3 3 3
8 mgm
39 mgm
15.71 ∓ 5.56
III. PRETREATED GH Rx (II)
2 3 4 6
17 mgm
Vehicle
29.00 ∓ 4.91
IV. POSTTREATED GH Rx (5)
Vehicle
3 3 3 3 3 3 3 3 3 3 3 3
39 mgm
5.00 ∓ 1.38
5 4 3 2 1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS

FIG. 5. Experiment 2. For explanation see text.

continuous therapy group, injected with growth hormone from the fifth day before through the twelfth day after inoculation; (3) *pretreated group*, injected with hormone from the fifth day before through the second day before tumor inoculation; and (4) *posttreated group*, received hormone from the first post-inoculation day, continuing through the twelfth postinoculation day.

The left-hand vertical line in Figure 5 represents the time of intravenous inoculation of a saline suspension of sarcoma T241 cells. The right-hand vertical line represents the time when all mice were killed and their surface lung tumors counted as in Experiment 1. These data, listed on the right-hand side of Figure 5, are in agreement with Experiment 1, since those animals which received hormone before tumor injection again showed a significant increase in the number of lung tumors developing. The evidence indicates that the more hormone the animals received before tail-vein inoculation the greater was the number of lung tumors seen. Further, while the pretreated groups received less than one-half the hormone administered to the posttreated group, the former had six times as many lung tumors as the latter group. The probability value between the control and the pretreated group was less than 0.01 and between the pretreated and the posttreated groups was also less than 0.01. Although the numbers in each group were relatively small, the chance likelihood of these differences was less than once in one hundred times.

Experiment 3. Mice were inoculated subcutaneously in the axilla with sarcoma T241 fragments and divided into two groups. After the fifth day of tumor

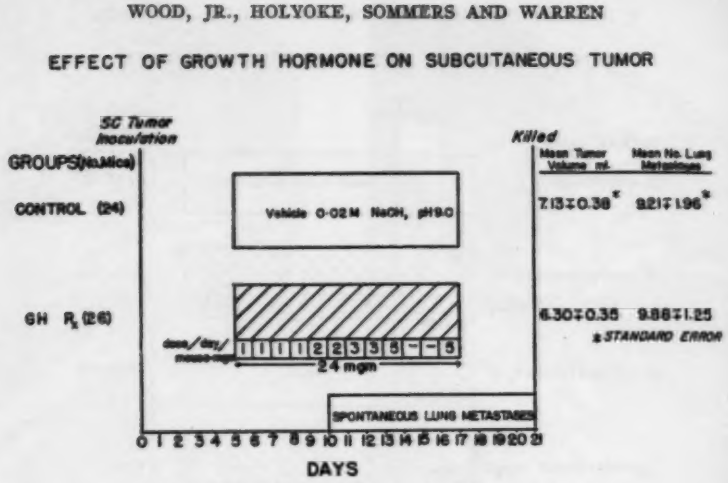
EFFECT OF GROWTH HORMONE ON SUBCUTANEOUS TUMOR
SC Tumor Inoculation
Killed
GROUPS(no.Mice)
Mean Tumor Volume ml.
Mean No. Lung Metastases
CONTROL (24)
Vehicle 0·02 M NaOH, pH 9·0
7·13±0·38*
9·21±1·96*
GH R1 (26)
6·30±0·35
9·88±1·25
* STANDARD ERROR
dose/day/mouse-mgm
1 1 1 1 1 2 2 3 3 3 – – 5
24 mgm
SPONTANEOUS LUNG METASTASES
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS

FIG. 6. Experiment 3. For explanation see text.

growth, these mice received daily subcutaneous injections of growth hormone (Fig. 6) as in Experiment 1. All animals were killed on the twenty-first day of tumor growth. At autopsy, in addition to counting lung metastases as before, the volumes of the primary tumors were determined by the method of fluid displacement (13). With the T241 sarcoma, lung metastases occur on and following the tenth day of tumor growth (14). The data recorded in Figure 6 show that growth hormone administered to mice already bearing a primary tumor had no effect on the size of the primary tumor or on the number of spontaneous lung metastases observed. It will be noted that the time of starting the administration of growth hormone, five days after subcutaneous inoculation, is still five days before metastases from this sarcoma ordinarily occur spontaneously.

Experiment 4. The chief contaminant of these growth hormone preparations, thyroid stimulating hormone (TSH), was injected subcutaneously into 6 animals from the fourth preinoculation day until the tenth postinoculation day, a total dosage of 7.9 mgm. per mouse. Four controls received an equivalent quantity of the vehicle (0.02 M NaOH, pH 9.0). All animals were killed on the thirteenth day, their lungs removed, and surface metastases counted as in

TABLE I

Effect of Thyroid Stimulating Hormone on Intravenous Tumor Suspensions

(Experiment 4. For explanation see text)

Group	No. Animals	Mean No. Lung Tumors
I. Controls	4	2.50 ± 1.55*
II. TSH	6	1.33 ± 0.61

* Standard error.

previous experiments. In Table I it is apparent that TSH did not increase the number of visible lung tumors resulting from tail-vein inoculation of tumor cell suspensions.

DISCUSSION

Pretreatment of the host with pituitary growth hormone increased the number and size of lung tumors following intravenous inoculation of tumor cells, while postinoculation treatment with the hormone was without effect. It would therefore appear in this instance that the effect of growth hormone was primarily on the host, rather than on the tumor *per se*.

In contrast, the failure of growth hormone, given during the period of spontaneous metastasis formation, to influence the frequency of spontaneous lung metastases in the animals bearing subcutaneous tumors would suggest that the exogenously administered growth hormone was then in excess, and that there already existed in an animal bearing a metastasizing tumor an altered constitution maximally favoring the survival and local growth of embolic cells. Exogenous growth hormone administered would thus be without effect.

The host alteration such as was seen following growth hormone administration, or as may occur in the presence of a primary metastasizing cancer, by permitting a relatively small increase in the number of successfully established emboli, would result in a large increment in the number of metastatic tumors observed in the lung. It is possible that an increased concentration of stromal cytoplasmic nucleic acids and an increase in cytoplasmic nucleoproteins in lung would represent one of the ways in which growth hormone may increase the essential nutrients for local tumor cell survival and growth, and therefore favor survival and growth of embolic tumor cells.

SUMMARY

Experiments have tested the effect of purified pituitary growth hormone on tumor growth and metastasis formation in mice with sarcoma T241, with the following results:

1) Growth hormone given to animals prior to and after the intravenous inoculation of a tumor-cell suspension increased the number of successful lung tumors and increased the rate of tumor growth.

2) Growth hormone given only after intravenous inoculation did not alter the number of lung tumors.

3) Growth hormone given after subcutaneous inoculation of tumor, but prior to and during the occurrence of spontaneous metastases, had no demonstrable effect on the primary tumor or its metastases.

4) Thyroid stimulating hormone, the only detected contaminant of the growth hormone preparations, did not alter the number of lung tumors, following the intravenous injection of tumor cells.

It is concluded from these studies, at least in the present case, that the effect of growth hormone is primarily on the host, promoting a constitutional alteration more favorable to the successful establishment and growth of embolic tumor cells.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the advice of Dr. I. Zeidman, the help of Miss Olive Lombard who aided with many of the statistical analyses, and the technical assistance of Miss Lilla Anderson and Mrs. Kathryn H. Haley.

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STUDIES ON THE MODE OF ACTION OF CITRATE THERAPY IN RICKETS*

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Received for publication October 12, 1954

In 1937 Shohl (1), and Hamilton and Dewar (2) reported that mixtures of citric or tartaric acid and their sodium salts, administered orally, were effective in the prevention and treatment of experimental rickets in the rat. Shohl and Butler (3) later found that the combination of citric acid and sodium citrate had a similar curative effect when given orally to two infants with rickets. These observations have since been confirmed by other investigators (4, 5, 6). Harrison and Harrison noted that microscopic evidence of healing can be demonstrated in rachitic rat cartilage as early as 24 hours after citrate therapy has begun (7).

The mode of action of the citric acid-sodium citrate mixture in rickets has not been apparent. Many years ago, Howland and Kramer (8) noted that if the Ca × P product‡ of the serum of children was below 30, rickets was invariably present and that healing did not occur until the Ca × P product was over 40. The curative action of vitamin D on the skeleton is accompanied by and is believed to be dependent upon, a rise in the levels of serum phosphorus§ and calcium. Hamilton and Dewar (2) noted somewhat higher concentration of the serum phosphorus *at the fifth and tenth day and at six weeks* in the animals treated with citrate and tartrate than in their control animals. However, when healing occurs in infants under the influence of citric acid-sodium citrate therapy, no significant rise in either the serum calcium or phosphorus has been demonstrated (3, 6). Shohl and Butler found that calcium fell and the inorganic phosphorus rose slightly (3), but the Ca × P products remained below 30. Thus, under citrate therapy, the skeletal lesions of rickets heal while the calcium and phosphorus levels of the serum are below those considered essential for the deposition of lime salts in bone.

* The original work described in this paper was carried out under a contract between the United States Atomic Energy Commission and The Johns Hopkins University.

† The studies herein reported were carried out during the tenure of a travelling fellowship from the R. Samuel McLaughlin Foundation. Dr. Yendt is currently at the Toronto General Hospital, Toronto, Canada.

‡ The term Ca × P product is used to denote the product of the calcium concentration expressed as mg. per 100 ml. × the concentration of inorganic phosphorus expressed as mg. per 100 ml.

§ The term "serum phosphorus" is used throughout this paper to refer to the inorganic phosphorus of the serum.

The following studies were undertaken in the belief that investigation of the manner in which rickets is healed under such peculiar circumstances might yield important information concerning the mechanisms of skeletal calcification.

Part I

In vitro calcification studies using cartilage from rachitic rats which were fed citrate prior to sacrifice

The fact that citrate therapy produces healing in rickets while the serum calcium and phosphorus remain at or near pre-treatment levels suggests that something has happened to improve the calcifiability of the cartilage and osteoid, i.e., so that they become calcified when the extracellular fluids contain less calcium and phosphorus than is ordinarily required for calcification. The following experiments were performed: Citric acid and sodium citrate were added to the diet of rachitic rats. Eight to 17 hours later, before calcification of the cartilage had occurred in vivo, as judged by microscopic study after death, the rats were sacrificed. Cartilage slices from each rat were then incubated in a series of inorganic solutions in order to determine the lowest concentrations of Ca × P which would produce calcification in vitro.* These results are compared with those obtained using cartilage from rachitic rats which had not been fed citrate.

The technique of in vitro calcification employed in these experiments has been described in detail in a previous communication (9). Male Wistar rats, three to four weeks old and weighing 40 to 55 gm., were fed a high calcium, low phosphorus rachitogenic diet (Rachitogenic diet #2 U.S.P. XII), plus distilled water for 20 to 30 days. At the end of this time when the rats were moderately rachitic, citric acid and sodium citrate were mixed with the diet so that the concentration of citric acid was 0.6 M. per kg. of feed mixture and that of sodium citrate was 0.4 M. per kg. of feed mixture.† The rats were sacrificed eight to 17 hours later. Because rats eat relatively little during the daytime, the new feed mixture was substituted in the evenings and the rats sacrificed the following morning. This resulted in the ingestion of adequate amounts of citrate prior to death. The rats were not kept in metabolic cages

* Ca × P products in these studies are not comparable to those of Howland & Kramer (8) previously mentioned. The latter figures refer to rachitic infants, whereas our results are obtained using cartilage from rachitic rats. Higher concentrations of Ca and P are necessary for calcification in the rat, which normally has a higher serum P than the human. In addition, Kramer's Ca × P products are obtained by using values for *total* serum calcium, part of which is "bound" to protein as an inactive complex, whereas in the inorganic solutions employed in these experiments all the calcium is presumably biologically "active".

† Each 100 gm. of this feed mixture was prepared as follows: Rachitogenic diet #2,75.6 gm., citric acid 12.6 gm. and sodium citrate 11.8 gm.

but it was estimated that each rat ate an average of 3 to 5 gm. of the feed which contained roughly 400 to 600 mg. each of citric acid and sodium citrate.

Longitudinal slices including a band of epiphyseal cartilage were made through the upper ends of both tibiae. Four or five suitable slices were usually obtained from each tibia. Slices were then incubated in a series of six solutions containing inorganic phosphate and calcium, also sodium, potassium, magnesium, chloride, bicarbonate and sulphate ions in concentrations approximating those of normal human serum. The phosphorus content of all solutions was 5.0 mg. per 100 ml. but the calcium concentrations differed. In each experiment the calcium concentration of the first solution employed was usually 3.5 mg. per 100 ml. and in each successive solution of the series it was increased by 0.5 mg. per 100 ml.* Thus cartilage slices from every rat studied were incubated in a series of six solutions, all containing 5.0 mg. inorganic phosphorus per 100 ml. but having calcium concentrations of 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mg. per 100 ml. (giving Ca $\times$ P products of 17.5, 20.0, 22.5, 25.0, 27.5 and 30.0). Following incubation in one of these solutions at 37.5°C for 18–24 hours, the slices were fixed, stained with silver nitrate and examined under the low power microscope for evidence of calcification in the epiphyseal cartilage. The degree of in vitro healing was graded 0 to 4 plus, according to the scheme used in a previous study (9). Three to four slices from each rat were not incubated in inorganic solutions but were instead fixed and stained with silver nitrate for control purposes. If the zone of epiphyseal cartilage in these control slices was too narrow because the rat was not sufficiently rachitic, or if any calcification was present in the epiphyseal cartilage indicating that healing had begun in vivo, the results were difficult to interpret and slices from such animals were not used in this study. We were usually unable to demonstrate any evidence of in vivo healing by these methods if the rats were sacrificed within 15 hours after citrate administration had begun.

The lowest Ca $\times$ P products that would calcify cartilage from control rachitic rats which had not been fed citrate prior to sacrifice were determined

* These solutions were made up from the following stock stolutions: 1. Double strength basal solution containing NaCl 12.0 gm. per litre, KCl 0.6 gm. per litre, $NaHCO_3$ 4.4 gm. per litre and $MgSO_4 \cdot 7H_2O$ 0.50 gm. per litre. 2. Solution of $NaKHPO_4$ (P conc. = 50 mg. per 100 ml.). 3. Solution of $CaCl_2$ (Ca. conc. = 70 mg. per 100 ml.). All stock solutions were sterilized by passage through an ultra-fine sintered glass filter (Corning #36060). To prepare each solution, 5.0 ml. of the doubel strength basal solution was used. The volume of $CaCl_2$ and $NaKHPO_4$ solutions necessary to give the desired concentrations of Ca and P in a volume of 10 ml. were calculated and then the volume of sterile distilled water necessary to bring the final volume to 10 ml. was also calculated. The P solution and distilled water were added to the basal solution first. The cartilage slices were then inserted and the pH adjusted to between 7.3 and 7.5 by shaking with 5% CO_2 in 95% O_2 for 3–4 minutes. The Ca solution was added last after pH had been adjusted, to avoid formation of a calcium phosphate precipitate.

TABLE I

Lowest Ca × P Products Producing In Vitro Calcification of Cartilage from Control Rats

Animal No.	Lowest Ca × P Product Giving In Vitro Calcification	Degree of In Vitro Calcification	Animal No.	Lowest Ca × P Product Giving In Vitro Calcification	Degree of In Vitro Calcification
1*	35.0	0	13	32.5	3+
2	35.0	3+	14	25.0	2+
3	31.0	4+	15	30.0	3+
4	37.5	4+	16*	32.5	0
5	37.5	1+	17	25.0	2+
6	37.5	3+	18	27.5	1+
7†	25.0	2+	19†	22.5	2+
8†	25.0	1+	20	25.0	2+
9	35.0	1+	21	27.5	1+
10	25.0	3+	22	30.0	1+
11	30.0	3+	23†	22.5	3+
12†	25.0	2+	24	25.0	3+

* In these experiments no calcification occurred in any of the solutions. The Ca × P product listed is that of the solution having the highest concentration of calcium.

† In these experiments, calcification occurred in all of the solutions. The Ca × P product listed is that of the solution having the lowest concentration of calcium.

in a like manner. However, the calcium concentrations of the solutions used for these control experiments were in a slightly higher range, i.e., usually from 5.0 to 7.5 mg. per 100 ml. in the first 12 control experiments and from 4.5 to 7.0 mg. per 100 ml. in the last 12. Both the control rats and those fed citrate were chosen at random from many different litters over a 9-month period. Some rats from a given litter might be used as controls and others fed citrate (or used for the other studies described in parts II and III).

The results using cartilage slices obtained from 24 rachitic rats which had not been fed citrate prior to death are given in Table I. Unfortunately, in two experiments, calcification did not take place in any of the solutions used and in five others it occurred in every solution used. Consequently, in these seven experiments the lowest Ca × P products which would produce in vitro calcification could not be determined. The lowest Ca × P products which calcified cartilage from the remaining 17 rats varied from 22.5 to 37.5. The significant finding was that in only 10 of 24 experiments did calcification occur in solutions having Ca × P products of 25.0 or less and in only two experiments did calcification occur in solutions having Ca × P products of 22.5 (although it is possible that cartilage from rats #7, 8 and 12 might also have calcified in solutions having Ca × P products of 22.5 had such been employed). In exactly one-half the control experiments, no calcification occurred until the Ca × P products of the bathing solutions was 30.0 or greater.

The results obtained using cartilage from 11 rachitic rats which had been fed citrate prior to sacrifice are given in Table II. In every instance, calcifica-

TABLE II

Lowest Ca × P Products Producing In Vitro Calcification of Cartilage from Rachitic Rats Fed Citric Acid and Sodium Citrate

Animal No.	Period of Citrate Therapy Prior to Sacrifice	Lowest Ca × P Product Giving In Vitro Calcification	Degree of In Vitro Calcification
	hrs.		
25*	10	25.0	3+
26*	11½	25.0	3+
27*	10½	20.0	3+
28*	11	25.0	4+
29	12	22.5	3+
30	11¼	22.5	3+
31	13	22.5	3+
32	15	20.0	2+
33	17	22.5	2+
34†	8	22.5	2+
35†	16½	20.0	2+

* In these experiments calcification occurred in all of the solutions. The Ca × P product listed is that of the solution having the lowest concentration of calcium.

† In these experiments procedure was varied slightly. Rat #34 received 120 mg. citric acid and 177 mg. sodium citrate in aqueous solution. This was dropped into the rat's mouth over a 4-hour period and the rat was sacrificed 8 hours after this was begun. Rat #35 received the same amount of citric acid and sodium citrate administered in a similar fashion over a 1-hour period. For the next 15 hours, the rat was allowed to eat the citric acid-sodium citrate feed mixture and was then sacrificed.

tion occurred in solutions having Ca × P products of 22.5 or less when solutions with such products were used (8 rats). In the remaining three experiments, calcification took place in solutions having Ca × P products of 25.0 but in these instances solutions having lower Ca × P products were not employed. It is quite possible that calcification would have occurred in solutions having Ca × P products of less than 25.0 in these experiments also, if such solutions had been used.

The foregoing results indicate that the administration of citric acid and sodium citrate *orally* to rachitic rats has an effect upon the hypertrophic epiphyseal cartilage whereby it undergoes calcification when bathed by solutions containing less calcium and inorganic phosphate than is ordinarily required for calcification.

Attempts were made to administer *parenterally* doses of citrate comparable to those which, when given orally, result in healing of experimental rickets. These attempts were unsuccessful owing to serious intoxication produced by the citrate in most of the animals. Though no deductions can be made from the following experiments, the negative findings are reported for the possible benefit of others engaged in similar types of study. It must be recalled that starvation will induce healing in experimental rickets, and therefore care must be exercised in the interpretation of healing induced by any substance which

produces sufficient intoxication to stop the animals from eating. It is our current belief that parenterally administered citrate will not of itself exert a healing effect on experimental rickets.

Rat #36, weighing 110 gm., was injected *subcutaneously* with 2.0 ml. of a 31.5% solution of citric acid and 2.0 ml. of a 29.5% solution of sodium citrate. This is roughly equivalent to the total amount of citric acid and sodium citrate *ingested* by each rat in the previous experiments. Within 10–15 minutes, the animal became extremely agitated and rushed furiously about the cage. Shortly thereafter, movements became clumsy and the rat had considerable difficulty in standing on its feet. Tetany supervened after 30 to 45 minutes and death in a convulsive seizure occurred approximately one hour after the injection. Slices were made immediately through the upper ends of the tibiae and incubated in a series of inorganic solutions having Ca × P products of from 15.0 to 30.0. No calcification occurred in any of these solutions.

Rat #37, weighing 140 gms., was injected subcutaneously with 30% of the amount of citric acid and sodium citrate given to rat #36 (0.6 ml. of each solution). Symptoms similar to those exhibited by the previous animal appeared but were much less severe. Tetany was just beginning when the rat was sacrificed 2½ hours after the injection. Cartilage slices were incubated in a series of inorganic solutions having Ca × P products of from 15.0 to 30.0. Again no calcification occurred in any of these solutions.

Rat #38, weighing 95 gms., was injected with 10% of the amounts of citric acid and sodium citrate given to the first rat (0.2 ml. of each solution). The animal was listless and lethargic for three hours after the injection but no tetany appeared. Four hours after the first injection, the injection was repeated using one-half the initial dose. The rat was then fed the usual rachitogenic diet and sacrificed 23 hours later. Cartilage slices were examined for evidence of "in vivo" healing but none could be demonstrated. The in vitro behavior of cartilage slices from this rat was not studied. In this experiment it was noted that necrosis of skin was present at the injection sites.

In an effort to avoid local tissue necrosis following the citrate injections, rats #39, 40 and 41 were each injected intraperitoneally with a 5.6% solution of citric acid and a 3.0% solution of sodium citrate. Such solutions are approximately isotonic and did not produce necrosis when so injected. Each rat was injected with 0.57 ml. of citric acid solution and 1.0 ml. of the sodium citrate solution every morning and evening for four days. (The total injected dose of citric acid being 255 mg. and of sodium citrate 240 mg.). Between injections the rats were fed the usual rachitogenic diet and the animals were sacrificed 96 hours after the initial injection. The epiphyseal cartilages at the upper ends of the tibiae of each rat were examined for evidence of healing "in vivo", but in no instance could such healing be demonstrated. In vitro studies using cartilage from these rats were not performed.

In none of the foregoing six experiments did the parenteral administration of citrate produce healing of rickets in vivo. In addition cartilage slices from two of these rats used for in vitro calcification studies did not calcify in solutions having lower Ca × P products than are usually requried. These findings suggest that citrate itself does not exert a direct beneficial effect upon rachitic cartilage but rather that its therapeutic action seems to be mediated via some mechanism in the gut. It is felt, however, that the experiments are too few in number to warrant drawing final conclusions. In addition, certain objections can perhaps be directed at the methods employed. In the first two experiments, it is possible that the time intervals between the citrate injections and death were too short to allow the citrate effect to be exerted. In the last three experiments, the interval between the institution of citrate therapy and death should have been sufficiently long to allow a therapeutic effect

to be exerted (for when citrate is given orally to the rachitic rat, healing can be demonstrated in considerably less than four days). However, in these instances the total dose of citric acid and sodium citrate was less than one-half that eaten in one day when the citrate was administered orally.

PART II

THE EFFECT OF INCREASING CITRATE CONCENTRATIONS IN SERUM AND INORGANIC SOLUTIONS UPON CALCIFICATION IN VITRO

The available clinical and experimental evidence suggests that the beneficial effect of citrate therapy in rickets is not due to an increased content of citrate in the serum. The serum citrate levels of three infants in whom healing of rickets was produced by citrate therapy were found by Harrison and Harrison (6) to remain normal at or about 2.0 mg. per 100 ml. Even if the citrate in the serum were increased, one would expect the calcifying ability of such serum to be decreased rather than increased because of the formation of an non-ionized calcium citrate complex (which would not be physiologically available or useful). This supposition is supported by the experimental findings of Shipley, Kramer and Howland (10), who noted that rachitic rats' cartilage did not become calcified in inorganic solutions when all the calcium was present as the citrate. Our own studies are in agreement with these findings. In Table III are given the results of adding increasing amounts of citrate to pooled infants' serum which had been shown to calcify rachitic rat cartilage. Good calcification occurred in 5.0 ml. of the serum and also in 5.0 ml. of the serum when diluted with 0.5 ml. of 0.9% saline. However, when citric acid was added in amounts sufficient to increase the serum citrate by 9.1 mg. per 100 ml. (the dilution factor remaining the same as for the saline), slightly less calcification occurred and when the serum citrate was increased by 18.2 and by 27.3 mg. per 100 ml., no calcification occurred at all.

TABLE III

Effect of Increasing Citrate Concentrations upon the Ability of Serum to Calcify Rachitic Rat Cartilage

Solution No.	Contents			Ca Conc.	P Conc.	Added Citrate	Degree of In Vitro Calcification
	Serum	0.9% NaCl	1% citric acid				
	ml.	*ml.*	*ml.*	*mg.%*	*mg.%*	*mg.%*	
1	5.0	0	0	10.9	5.0	0	4+
2	5.0	0.50	0	9.8	4.6	0	4+
3	5.0	0.45	0.05	9.8	4.6	9.1	3+
4	5.0	0.40	0.10	*	*	18.2	0
5	5.0	0.20	0.30	*	*	27.3	0

* Ca and P determinations were not done. Since the dilution factor in these solutions was the same as in solutions 2 and 3, the Ca and P concentrations were also presumably the same, i.e., Ca = 9.8 mg. % and P = 4.6 mg. %.

TABLE IV

Effect of Increasing Citrate Concentrations upon the Ability of Inorganic Solutions to Calcify Rachitic Rat Cartilage

Solution No.	Vol.	Ca Conc.	P Conc.	Ca × P Product	Citrate Conc.	Degree of In Vitro Calcification
	ml.	mg.%	mg.%		mg.%	
1	10	5.5	4.8	26	0	2+
2	10	7.3	4.8	35	0	4+
3	10	7.8	4.8	37	0	3+
4	10	9.0	4.8	43	15.0	3+
5	10	9.0	4.8	43	17.5	3+
6	10	9.0	4.8	43	20.0	3+
7	10	9.0	4.8	43	22.5	0
8	10	9.0	4.8	43	25.0	0

Similar results were obtained when citric acid was added to inorganic solutions (Table IV). In this experiment, cartilage slices from one rachitic rat were incubated in a series of eight inorganic solutions. The first three solutions had Ca × P products of 26, 35 and 37 and contained no citrate. Good calcification occurred in all these solutions. The Ca × P product of the remining five solutions was 43 but in addition citric acid was present in concentrations of 15.0, 17.5, 20.0, 22.5 and 25.0 mg. per 100 ml. No calcification occurred when the concentration of citrate was higher than 20 mg. per 100 ml.

Part III

Results of injecting phosphate into rachitic rats

The foregoing studies have shown that the administration of citric acid-sodium citrate mixtures to rachitic rats alters the cartilage in such a way that it will calcify in solutions having lower Ca × P products than those ordinarily required for calcification. Other evidence indicates that this effect is probably not dependent upon a rise in serum citrate levels.

In 1923 Pappenheimer showed that parenteral administration of phosphate would prevent the appearance of experimental low phosphorus rickets (11). Therefore, the following experiments were carried out to see if injections of inorganic phosphate would *heal* experimental rickets and in so doing would produce the same sort of functional alterations in the cartilage as those which citric acid-sodium citrate mixtures have been shown to produce—i.e., that cartilage would be calcifiable in inorganic solutions having lower Ca × P products than those usually necessary for calcification.

In these experiments, 13 rachitic rats were injected intraperitoneally with an isotonic solution of sodium acid phosphate and disodium phosphate. The pH of this solution was 7.3 and the total phosphorus concentration 310 mg. per 100 ml. The amounts of phosphate injected and the interval of time between

TABLE V

Effects of the Parenteral Administration of Inorganic Phosphate upon the Cartilage of Rachitic Rats*

Animal No.	Weight	Vol. of P Solution Injected	Mg. P per Kg. Animal Weight	Interval between Injection and Sacrifice of Animal	Results
	gm.	*ml.*		*hours*	
42	60	4.5	233	—	Rat found dead in cage 20 hrs. later. Bones not examined
43	60	3.0	155	29	Considerable healing (2–3+) had occurred in vivo
44	105	5.0	143	—	Rat died in tetany 5 hrs. later. 1+ calcification of cartilage had occurred in vivo
45	110	4.0	113	4	1+ calcification in vivo
46	55	3.0	169	4	2+ calcification in vivo
47	58	3.0	160	3	1–2+ calcification in vivo
48	115	4.0	108	2	No calcification in vivo. In vitro calcification occurred with Ca × P product of 15.0
49	115	4.0	108	2	No calcification in vivo. In vitro calcification occurred with Ca × P product of 17.5
50	85	3.0	106	1½	No calcification in vivo. In vitro calcification with Ca × P product of 22.5
51†	120	4.0	103	2	No calcification in vivo. In vitro calcification with Ca × P product of 22.5
52†	130	4.0	95	5½	No calcification in vivo
53	87	1.0	36	7	? early calcification in vivo
54	70	1.0	44	4½	2+ calcification in vivo

* As an isotonic solution of NaH_2PO_4 and Na_2HPO_4 containing 310 mg. P per 100 ml.
† Sacrificed with ether.

the injections and sacrifice differed from rat to rat (Table V). Cartilage slices were obtained from the upper ends of both tibiae as previously described. The slices from one tibia were kept as controls and subsequently stained with silver nitrate to see if healing had occurred in vivo. Slices from the other tibia were incubated in previously prepared inorganic solutions to determine the lowest Ca × P product which would produce calcification in vitro. If in vivo healing was found in the control slices, the in vitro studies could not be interpreted and were discarded.

The results are given in Table V. Eight rats which received 36 to 155 mg. phosphorus per kg. of body weight were sacrificed three or more hours after the injection. All but two of these rats showed definite evidence of healing in vivo. Of the two exceptions, the one which received the smallest dose of phosphate (36 mg. per kg.) showed questionable evidence of healing after seven hours. The other exception received 95 mg. P per kg. and showed no evidence of in vivo healing when sacrificed five hours later. Four rats which received doses of 103–108 mg. P per kg. of body weight were sacrificed within two hours after

TABLE VI

Rise in Serum Phosphorus Soon After Rachitic Rats Are Fed Citrate with the Diet

Animal No.	Period of Citrate Feeding Prior to Sacrifice	Serum Phosphorus at Time of Sacrifice
	hours	mg.%
55	0*	2.9
56	3	4.0
57	3	4.1
58	6	5.9
59	15	5.9
60	19	5.5

* Control.

the injections. No in vivo healing was detectable in any of these rats. In every instance, cartilage slices from these animals underwent calcification in inorganic solutions having Ca × P products lower than those usually found necessary to calcify cartilage from control rats—i.e., 15.0, 17.5, 20.0 and 22.5.

OBSERVATIONS ON SERUM PHOSPHORUS SOON AFTER ADMINISTRATION OF CITRATE MIXTURE

The changes induced in the rachitic cartilage by parenteral injection of phosphate are so similar to those induced by oral administration of citrate that the possibility immediately comes to mind that the mechanism which effects the healing of rickets may be the same in both instances. The modus operandi of phosphate administration is believed to be elevation of the serum phosphorus concentration. No such *immediate* rise in serum phosphorus has been demonstrated following citrate therapy in either infantile or experimental rickets. However, it was felt that, in the past, serum chemical determinations might not have been made soon enough after therapy was begun to detect such a rise. The following experiments were performed to investigate this point. Six rats from the same litter were fed the standard rachitogenic diet for three weeks. At this time, blood for serum phosphorus determination was obtained from one rat and the remaining five were then placed on the same citrate feed mixture as used for the experiments described in Part I. At intervals of 3, 6, 15 and 19 hours later, the animals were anesthetized with ether, and blood for serum phosphorus determinations was obtained by aortic puncture (Table VI). The results indicated that, shortly after beginning citrate therapy, there occurred a rise in serum phosphate which was of sufficient magnitude to initiate the healing of rickets, provided other factors remained unchanged.

Another and more extensive series* of animals were then tested in similar manner. Twenty-four weanling male rats were placed on the rachitogenic diet

* We are indebted to Dr. William C. Thomas, Jr., who carried out these studies after Dr. Yendt's tenure of fellowship had ended.

TABLE VII

Effect on Serum Phosphorus of Citrate Feeding to Rachitic Rats

Period of Citrate Feeding Prior to Sacrifice	Serum P of Control Rats	Serum P of Citrate Fed Rats	Time of Day
hours	*mg.%*	*mg.%*	
0	1a = 5.6		4 p.m.
	1b = 2.3		4 p.m.
6	2a = 3.7	2c=qns	10 p.m.
	2b = 3.0	2d=7.2	10 p.m.
15	3a = 3.1	3c=4.3	7 a.m.
	3b = 3.7	3d=5.7	7 a.m.
19	4a = 3.3	4c=3.7	11 a.m.
	4b = 3.6	4d=4.3	11 a.m.
24	5a = 2.7	5c=6.3	4 p.m.
	5b = 2.2	5d=9.2	4 p.m.
30	6a = qns	6c=7.9	10 p.m.
	6b = 1.1	6d=4.8	10 p.m.
48		7c=qns	
		7d=7.2	

and, after 26 days, citrate therapy was begun to one-half of them. Two animals were sacrificed at each of the following intervals of time after initiation of the citrate feeding: 6 hours, 15 hours, 19 hours, 24 hours, 30 hours and 48 hours. Two control (not citrate treated) rats were sacrificed at each period. Results are shown in Table VII.

In two of the citrate treated rats insufficient serum could be obtained for determination of phosphorus. All the remainder disclosed serum P of 3.7 mg.% or higher, and most of them were considerably higher. In the control (non-citrate treated) rats, the serum P was 3.7 or below with but a single exception (rat 1A) which was very small and showed minimal evidence of rickets by x-ray, presumably because it had eaten less well than the others. These results corroborated the finding in the previous series, that a significant rise in serum phosphorus soon follows the addition of citrate mixture to the diet of rats made rachitic by the high calcium, low phosphorus diet (U.S.P. XII, Rachitogenic Diet #2).

DISCUSSION

During the many years in which efforts have been made to understand the mechanisms whereby the skeletal tissues normally calcify, many experimental observations have been made on the environmental requirements needed for

rachitic cartilage to calcify in vitro. Since Howland and Kramer originally noted that the gross anatomical and histological changes of rickets did not occur in children unless concentrations of the serum calcium and phosphorus were lowered (8), it has been shown repeatedly that experimental rickets likewise is not produced unless there is reduced concentration of either calcium, phosphorus or both in the serum of animals. Also in vitro calcification of rachitic rat cartilage will not occur unless the concentration of these constituents in the bathing media is sufficiently high. Despite the purely empirical nature of the Ca $\times$ P product, there seems no doubt that there is a point for each species below which serum concentrations of Ca and P cannot be reduced in *otherwise normal young animals* without production of the lesions of rickets, and that above such concentrations the cartilage will calcify either in vivo or in vitro. Other factors in the calcification mechanism are of course necessary; Robison used the term "unknown factor" for an undefined quality of the matrix, and McLean has called it "factor X".*

It was, therefore, of considerable interest to note that addition of citrate to the diet resulted in healing of rickets without an apparent coincident rise of either the serum calcium or phosphorus. The natural assumption followed that the curative action of citrate might somehow have operated by a priming action on the "unknown factor" or "factor X". And, in so far as they go, the experimental findings do point toward some local change resulting from citrate, so that in vitro (and presumably in vivo) calcification will now occur in the presence of serum calcium and phosphorus (Ca$\times$ P product) lower than would be required for calcification without citrate therapy. The experiments do not disclose, however, *what* the citrate has done to effect this altered receptivity to lime salt deposition. The finding that very brief intraperitoneal phosphate administration will also effect a change such that the cartilage will calcify in vitro with less than the usually required concentrations of calcium and phosphorus in the environment, and the observation that early in citrate therapy the serum phosphorus rose sharply, would seem to indicate that the "priming" of the mechanism had occurred coincident with concentrations of calcium and phosphorus which would ordinarily be conducive to calcification and rachitic healing.

Of the current concepts, the theory of initiation of the calcifying mechanism proposed by Neuman (12) seems to explain best these observations. Neuman's theory holds that skeletal calcification is a process of crystallization of salts on a preformed organic surface, the phosphate radicles of which are systematically arranged on the collagen fibres in the spatial configuration of the bone salt

* For a discussion of the known facts regarding calcification mechanisms in vitro, the reader is referred to the Trans. 5th Conference on Metabolic Interrelations, Josiah Macy, Jr. Foundation, New York, Jan. 5–6, 1953, p. 11.

lattice. Calcium and then phosphate are deposited alternately on this basic template (along with smaller quantities of other ions such as citrate, hydroxyl, etc.) to form bone salt. On the basis of such a theory, it is possible and even likely that, *once crystallization on the basic template had begun*, it would continue even though the concentrations of calcium and phosphorus in the bathing fluids were to become lower than those which had originally been necessary for the initiation of the process. For this theory to fit in with the observations reported in this paper, it would have to be postulated that cartilages from our experimental animals treated briefly with citrate or phosphate had already begun the process of calcification, but that at this stage such calcification could not be detected by the Von Kossa silver nitrate technique.

During discussions with Dr. Neumann (13) of the data presented in this communication, he pointed out that the quantity of the citrate in the diet which promoted healing was sufficient to "complex" nearly all the calcium present, so that most of the phosphate would be free to be absorbed instead of being precipitated as calcium phosphate (a suggestion also made by Hamilton and Dewar (2) as one of several possible explanations for the healing of rickets by citrate and tartrate). A sudden increase in absorbed phosphate would result in a sharp rise in serum phosphate, sufficient for initiation of the calcification process. Once begun, the removal of salts for continuing crystal deposition would reduce the serum calcium and phosphorus concentrations back to or perhaps even lower than the original levels. If this thesis of Neuman's is correct, balance data on patients or animals during healing of rickets under citrate therapy should disclose sharply increased absorption of phosphate and probably of calcium as well. If the theories of Neuman correctly interpret the events of the experiments reported here, adequate quantities of other calcium-complexing agents, if added to the usual experimental diets for producing rickets (high calcium, relatively low phosphorus) or even a sudden change to low calcium diet with similar phosphorus content, should likewise permit calcification to commence. On the other hand, true *low calcium* rickets, difficult to produce in the experimental animal, should not respond nearly so well to citrate therapy. Data on these points will be awaited with much interest.

SUMMARY

Addition of mixtures of citric acid and sodium citrate to the diet have been shown to result in healing of experimental and naturally occurring rickets, and during such healing calcium and phosphorus concentrations in the serum have been reported to remain below normal—i.e., in the range in which rickets is ordinarily produced.

Experiments are here reported in which cartilage slices were obtained from rachitic rats which had been fed for brief periods on citrate mixture and before any healing was discernible under the microscope. These slices were compared

with slices from untreated rachitic rats as to their calcifying properties when tested in prepared solutions of calcium and inorganic phosphate. It was found that the cartilage of the citrate treated animals calcified in media of significantly lower calcium and phosphate concentrations than did the cartilage of the control animals.

Similar experiments were made using cartilage from animals to which phosphate had been administered intraperitoneally, and before any evidence of healing could be detected. The cartilage of the phosphate treated animals also calcified in solutions of lower calcium and phosphate ion concentrations than did the cartilage from the control animals.

A series of rachitic rats were fed the citrate mixture and sacrificed at periodic intervals during the first 24 hours after therapy had begun. Serum obtained from these animals disclosed an appreciable rise in phosphorus concentration, and this rise is believed to be sufficient to account for initiation of the healing process.

The data suggest that, once the process of calcification has commenced, crystals can continue to be deposited despite concentrations of calcium and phosphate which would be too low to initiate the process. An explanation of this phenomenon is offered, based on the theories of biological calcification suggested by Neuman.

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CUSHING'S SYNDROME WITH BILATERAL ADRENAL HYPERPLASIA: A STUDY OF THE PLASMA 17-HYDROXYCORTICOSTEROIDS AND THE RESPONSE TO ACTH[1]

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Since the original description of Cushing's syndrome (1) in 1932, the clinical picture, the course of the disease, and the pathologic physiology have been clarified considerably by numerous investigators (2, 3, 4). However, laboratory methods designed to measure the production of steroid hormones have not been uniformly diagnostic in the recognition of this disorder (5, 6, 7, 8). This may be attributed in part to the variability of the disease and the frequent wide daily fluctuations in the levels of adrenocortical metabolites in the urine. There are also limitations to the confidence which may be placed in the specificity of many methods presumed to detect these compounds in biological fluids (9).

The recent improvements in laboratory techniques, particularly those for the quantitative measurement of 17-hydroxycorticosteroids in circulating blood (10), have contributed greatly to the more precise evaluation of adrenocortical function. In our laboratory the plasma levels of free (unconjugated) steroids obtained at approximately the same time of day have shown fluctuations, so that a single determination may not be helpful.

The present study was designed to clarify some of the factors in the interpretation of laboratory tests in Cushing's syndrome and to develop systems whereby this condition may be more readily differentiated, especially in patients with many of the clinical features of the disease who have normal or equivocal laboratory findings by the present methods.

METHODS AND MATERIALS

Studies of plasma 17-hydroxycorticosteroids are reported on four patients with Cushing's syndrome, in three of whom there was proven bilateral adrenal hyperplasia, and in one metastatic adrenocortical carcinoma. These are compared to the findings in thirteen patients with virilizing adrenal hyperplasia as defined by Wilkins (11), one with a virilizing adrenocortical tumor, four with Addison's disease, and a number of subjects with presumably normal adrenal function.

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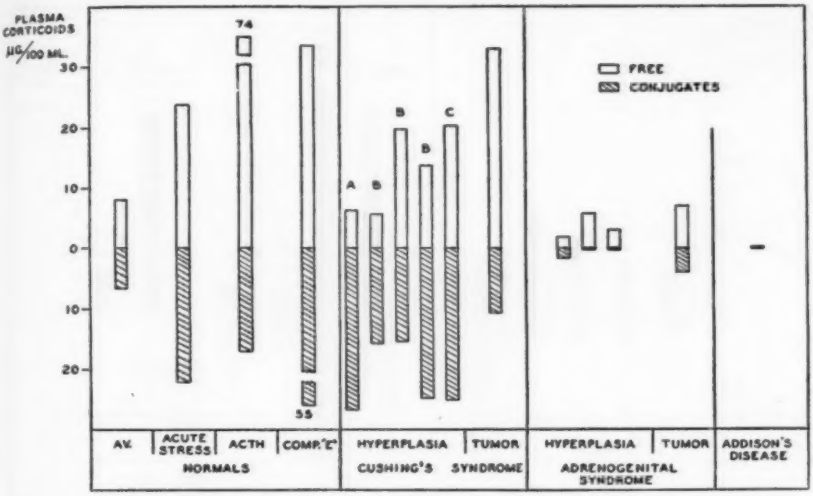

FIG. 1. The plasma levels of "free" and "conjugated" 17-hydroxycorticosteroids (Porter-Silber chromogens) in 4 patients with Cushing's syndrome. (Cases A and B are summarized in the text (addendum); Case C is a 11 year old girl with verified adrenal hyperplasia and Cushing's syndrome studied at the National Institute of Health through the courtesy of Dr. Roy Hertz. The value for adrenocortical tumor was obtained in a 6 year old girl, under the care of Dr. Harold Harrison at the Baltimore City Hospital, in whom extensive hepatic metastases were found at autopsy). For comparison the mean values of subjects with presumably normal adrenal function are shown. The level for acute stress is an average of 4 determinations in patients with severe infection, trauma, or immediately following major surgery. "ACTH" indicates the level in a 6 year old boy who had received 40 units of Armour ACTH in gelatin daily for the preceding 3 weeks. "Comp. E" shows the level in a normal individual 1 hour after the oral administration of 100 mg. of cortisone. The results in patients with the adrenogenital syndrome and in one patient with Addison's disease are compared.

Blood was drawn between 9 A.M. and 10 A.M. in a heparinized syringe and the plasma promptly separated. Plasma samples were extracted, chromatographed on specially prepared florisil, and treated with phenylhydrazine in dilute sulfuric acid according to the method of Nelson and Samuels (10) for the determination of "free" 17-hydroxycorticosteroids (Porter-Silber chromogens).[4] The residual plasma was incubated with beta-glucuronidase[5] after adjusting the pH to 4.5 with acetate buffer, and the "conjugated" 17-hydroxycorticosteroids (Porter-Silber chromogens) released by the enzyme were measured by the technique described by Bongiovanni (12, 13). Unless otherwise specified, responsiveness to

[4] Although the Porter-Silber reaction measures pure 17,21 dihydroxy-20-ketosteroids quantitatively, other substances, as yet unidentified, extracted from plasma and urine also may react with this reagent, despite chromatographic fractionation of such extracts. As a note of caution Dr. Harold Mason has suggested the term Porter-Silber chromogen.

[5] Ketodase, beef liver β-glucuronidase, was obtained from the Warner-Chilcott Laboratories, New York.

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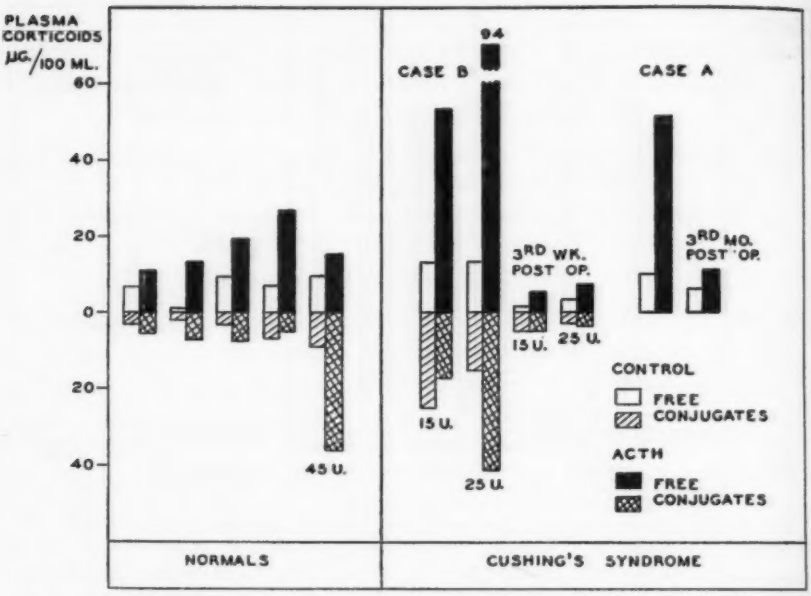

FIG. 2. The responses of the plasma 17-hydroxycorticosteroids (Porter-Silber chromogens) to a 4 hour intravenous infusion of ACTH in 5 subjects with normal adrenal function and in 2 patients with Cushing's syndrome associated with bilateral adrenal hyperplasia prior to and following subtotal adrenalectomy. The conjugates were not determined in Case A. The first bar for each individual is the control level and the second bar the value after ACTH.

ACTH was determined after the intravenous administration over a 4 hour period of 0.25–0.5 units/kg. of Armour ACTH[6] in 200 ml. of saline.

The clinical abstracts of two patients with Cushing's syndrome associated with bilateral adrenal hyperplasia, in whom plasma 17-hydroxycorticosteroids were determined prior to and following radical subtotal adrenalectomy, are presented in the addendum.

RESULTS

a) *Plasma levels*

Figure 1 shows the level of free and conjugated plasma 17-hydroxycorticosteroids in normal individuals and in patients with Cushing's syndrome, the adrenogenital syndrome, and Addison's disease. In this laboratory the mean value of the free plasma 17-hydroxycorticosteroids of normal individuals is 8.0 ± 1.0µg./100 ml. (range 2–10 µg.) and of the conjugated moiety 6.5 ± 3.0 µg./100 ml (range 0–11.9µg.). Under conditions of acute stress or on the prolonged intramuscular administration of long acting ACTH both the free and conjugated corticoids were found to be considerably increased. One hour after

[6] Lot no. RJ 21812. We wish to thank the Armour Co. for contributing the ACTH.

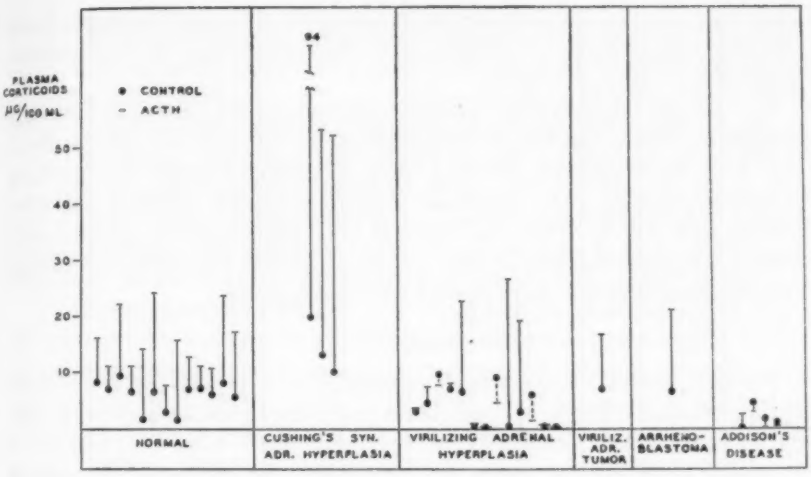

FIG. 3. Indicates the increase of the plasma "free" 17-hydroxycorticosteroids (Porter-Silber chromogens) following the intravenous administration of ACTH in a group of normal subjects, patients with various adrenal disorders, and in a patient with an arrhenoblastoma of the ovary. The dot indicates the initial level and the bar the value after ACTH. The broken line denotes a fall after ACTH.

the oral administration of 100 mg. of cortisone to a normal subject both fractions were increased. The studies on three patients with verified Cushing's syndrome associated with adrenal hyperplasia (A, B, C) showed that at times the free 17-hydroxycorticosteroids were not elevated above the levels of normal individuals. When, however, the conjugated corticoids were determined in addition, the total amounts were always increased. In the patient with Cushing's syndrome due to an adrenocortical carcinoma possibly the comparatively small percentage of the conjugated fraction was due to impairment of hepatic conjugation, resulting from extensive liver metastases found at autopsy.

In contrast to the findings in Cushing's syndrome, low values of free 17-hydroxycortisteroids and low or unmeasurable levels of conjugates were found in three cases of the adrenogenital syndrome due to adrenal hyperplasia[7], while normal amounts were present in the patient with a virilizing adrenal tumor. The patient with Addison's disease had no measurable corticoids.

b) *Response of the plasma 17-hydroxycorticosteroids to intravenous ACTH*

Figure 2 shows the responses of the plasma 17-hydroxycorticosteroids to a 4 hour intravenous infusion of ACTH in 5 normal subjects and in 2 patients with Cushing's syndrome associated with bilateral adrenal hyperplasia. In the

[7] The levels of free plasma corticoids are generally low in virilizing adrenal hyperplasia but normal values may be obtained in some patients (see Fig. 3).

latter the large rises before operation are compared to the small responses found following radical subtotal adrenalectomy. (In Case A only the free corticosteroid moiety was determined.)

Figure 3 also illustrates the hyperresponsiveness of the adrenal in Cushing's syndrome due to hyperplasia in comparison to the responses in normal individuals. It shows, in addition, that only three of thirteen patients with virilizing adrenal hyperplasia had a significant increase of plasma corticosteroids after infusion with ACTH. There was minimal or no response in the patients with Addison's disease. One patient with a virilizing adrenal tumor and one with an arrhenoblastoma of the ovary showed a normal response.

DISCUSSION

The daily variation in the *urinary* excretion of 17-ketosteroids and corticoids and the occasional failure of these values to deviate significantly from the normal in patients with Cushing's syndrome, particularly when the cause is not carcinoma of the adrenal cortex, has complicated the laboratory diagnosis of this disorder. When the disease is mild or the clinical picture equivocal, the diagnosis and institution of early therapy may be delayed.

The results of this study indicate that the level of "free" plasma 17-hydroxycorticoids on occasions may be within the limits of normal in Cushing's syndrome with bilateral adrenal hyperplasia. However, in these instances the "conjugated" plasma 17-hydroxycorticoids were elevated and the determination of both the free and conjugated steroid moieties provided a more complete estimate of the corticosteroids in human plasma. This might indicate that the plasma corticoids may be so rapidly conjugated in some patients that the major portion may not be detected by procedures which measure only the free fraction. Previous investigation (13) has demonstrated that pregnane-3α, 11β, 17α, 21 tetrol-20 one, tetrahydro F, is the major α ketolic component of the conjugated fraction in the normal individual. Although the conjugates are hormonally inactive, nonetheless they are an index of adrenocortical activity as evidenced by the rise following the administration of ACTH and cortisone, and the high levels which may be obtained during periods of acute stress.

The response of the plasma 17-hydroxycorticosteroids to the intravenous administration of ACTH is sharply contrasted in the various adrenal disorders. Comparison with the results in virilizing adrenal hyperplasia and Addison's disease, disturbances in which the pathogenesis is better understood, may provide a clue to the underlying defect in Cushing's syndrome. In Addisonian patients little or no rise occurs. Probably if a remnant of functioning adrenal tissue is present, it is already synthesizing 17-hydroxycorticosterone at a maximal rate under the stimulus of endogenous ACTH in an effort to maintain homeostasis. Increased levels of blood ACTH have been found in this disease

(14). In virilizing adrenal hyperplasia it is postulated that there is an enzymatic block in the biosynthesis of C 21 steroids by the adrenal cortex so that there is difficulty in the conversion of 17-hydroxyprogesterone to 17-hydroxycorticosterone according to the Hechter hypothesis of adrenal corticosteroid biogenesis (15). The evidence in favor of this concept has recently been presented (16, 17, 18). The blood levels of ACTH are high in this disorder (19). The administration of ACTH failed to cause a rise in the plasma corticoids in 10 of 13 patients with this disease. The significant rise in 3 of the patients suggests that varying degrees of impairment of 17-hydroxycorticosterone synthesis may occur.

In Cushing's syndrome with adrenal hyperplasia we obtained a brisk rise of the plasma 17-hydroxycorticoids following the intravenous infusion of ACTH. This hyperresponsiveness disappeared after radical subtotal adrenalectomy. Our findings are in conformity with the recent observations of Laidlaw and his associates (20) and Burnett and his co-workers (21), that exogenous ACTH causes excessive increases in the output of urinary 17-hydroxycorticoids in similar cases.These workers found that in the presence of an adrenal adenoma with Cushing's syndrome the rise in urinary corticosteroids was variable; in some patients the response was normal while in others an abnormal rise was demonstrated. The effect of ACTH appears to depend upon the degree of autonomy of the tumor and possibly the ability of ACTH to stimulate the contralateral adrenal. In adrenocortical carcinoma no rise occurred. Knowlton, Pool, and Jailer have failed to observe a significant change in the urinary excretion of corticoids and 17-ketosteroids following complete hypophysectomy in a patient with metastatic adrenocortical carcinoma associated with Cushing's syndrome (22).

There has been considerable discussion as to whether Cushing's syndrome accompanied by adrenal hyperplasia is due to a disorder of the adrenal cortex, or to excessive stimulation by ACTH caused by a pituitary-hypothalamic disturbance. Although subtotal adrenalectomy offers the best chance of "cures" (5, 23), it seems clear that pituitary irradiation (3, 24–26) or partial electrocoagulation of the hypophysis (27) may cause temporary or permanent remissions in some patients.

The measurement of the content of ACTH in the blood of patients with Cushing's syndrome should clarify the problem considerably. Unfortunately the results of assay of blood corticotropic activity from various laboratories (14, 28, 29) have been contradictory and until more definite data are available no final conclusions can be drawn. Sydnor and her associates (14), and Paris et al. (28) have failed to detect any ACTH in the blood of normal individuals or cases of Cushing's syndrome, although Sydnor observed increased levels in patients with Addison's disease (14) and virilizing adrenal hyperplasia (19). Utilizing another method of extraction and a modification of the assay, Gray

and Parrot (29) found elevated levels in three out of four cases of "Cushing's syndrome" and detectable quantities in normal subjects. The role of the hypothalmus and other portions of the central nervous system in the regulation of ACTH secretion has been reviewed by Porter (30). The likelihood of a disturbance of this regulatory mechanism in Cushing's syndrome is a matter of conjecture.

The significance of the finding of increased responsiveness of the adrenal to exogenous ACTH is not clear. Hyperresponsiveness would seem unlikely if the adrenals were already functioning under increased stimulation by ACTH from the pituitary. Furthermore, one might expect that increased production of adrenal corticoids would inhibit the secretion of ACTH. However, other possibilities might be that in Cushing's syndrome there is primarily an intrinsic abnormality in the enzymatic biosynthesis of C 21 steroids or that chronic ACTH stimulation has altered enzyme systems effecting the formation of corticosteroids.

There are some studies which give credence to these possibilities. Hechter and his co-workers (15, 31) recently have demonstrated, on the basis of perfusion experiments in isolated bovine adrenal glands, that ACTH increases corticosteroid synthesis by accelerating the rate of conversion of cholesterol to progesterone. An alternative pathway which utilizes acetate and other as yet unidentified precursors for conversion to C 21 steroids is relatively unaffected by ACTH. Dorfman (32) has obtained an enzyme "20,22 desmolase" from adrenal homogenates which facilitates the side chain cleavage of cholesterol between carbon 20 and 22, thus forming a C 21 steroid. An explanation of the hyperresponsiveness to ACTH of patients with Cushing's syndrome due to adrenal hyperplasia might be that there is an alteration of this enzyme system because of an intrinsic disturbance in the adrenal cortex or through the effects of chronic hypersecretion of ACTH. This may account for the abnormally high rate of corticosteroid formation as compared to the generally normal or only moderately elevated excretion of 17-ketosteroids found in these patients (33). Kass and his associates (34) have shown that chronic ACTH administration changes the pattern of corticosteroid secretion in the rabbit adrenal, which normally produces primarily corticosterone with a very low ratio of hydrocortisone to corticosterone as compared to the human (35). The daily injection of ACTH for 3 to 4 weeks caused a striking increase in the production of hydrocortisone and the ratio of hydrocortisone to corticosterone. This suggested that prolonged ACTH stimulation had selectively increased the 17-hydroxylating enzyme system in these adrenal glands. Studies on the qualitative and quantitative nature of the enzymes involved in corticosteroidogenesis in the adrenals of patients with Cushing's syndrome and virilizing adrenal hyperplasia should greatly further our understanding of these disorders.

CONCLUSIONS

Some of the difficulties in establishing a laboratory diagnosis of Cushing's Syndrome have been discussed. The value of estimating the conjugated as well as free circulating 17-hydroxycorticosteroids to obtain a more complete appraisal of adrenocortical activity is indicated.

In two patients with Cushing's syndrome associated with bilateral adrenal hyperplasia a hyperresponsiveness to intravenous ACTH was demonstrated. This was evidenced by an abnormal rise in the plasma 17-hydroxycorticosteroids. The results are compared and contrasted to those obtained in the same patients after subtotal adrenalectomy and in cases of the adrenogenital syndrome and Addison's disease. The importance of this test and the possible mechanism of the pathological response in Cushing's syndrome is described.

ADDENDUM

Case A

D. W. (J.H.H. Hist. No. 699914), a 20 year old married woman, was admitted to the Johns Hopkins Hospital in April, 1954 with a typical picture of Cushing's syndrome. The visual fields were normal. No abnormality of the sella turcica was visible in the roentgenograms of the skull. The urinary 17-ketosteroids varied from 19–34 mg/day and the 17-hydroxycorticoids from 26–35 mg/day (normal 2–7 mg). In May, 1954 a radical subtotal adrenalectomy was performed. The entire right adrenal (weight 13 gm.) and at least 85 per cent of the left adrenal (weight 12 gm.) were removed. Both adrenals appeared hyperplastic. Subsequently the signs of Cushing's syndrome completely disappeared and three months following operation the patient became pregnant.

Case B

P. S. (H.L.H. Hist. No. A 48266) was first seen at the Harriet Lane Home in August, 1951, at the age of 10 years with signs of Cushing's Syndrome. The sella turcica was normal on roentgenograms of the skull; no visual field defects were present. The urinary 17-ketosteroid excretion averaged 10.9 mg/day and no change occurred after the intramuscular administration of 50 mg. of cortisone daily for 16 days. The urinary corticoids by the method of Heard and Sobel were normal. In October, 1951 on exploratory laparotomy both adrenal glands were found to be hyperplastic in appearance. Subsequently, the patient received a course of pituitary irradiation which resulted in a remission of the disease for the following two years. In February, 1954, an exacerbation occurred with rapid progression of symptoms over the next few months. As a result, a radical subtotal adrenalectomy was performed in July, 1954 and the entire left adrenal gland (weight 10.2 gm.) and about 90 per cent of the right adrenal (weight 8.5 gm.) were removed. In the months following operation all evidence of Cushing's syndrome subsided. At present 7 months after surgery the patient is well and does not require cortisone replacement therapy.

ACKNOWLEDGEMENTS

We are indebted to Mrs. Betty Lawrence for technical assistance and for the preparation of the charts.

We owe our gratitude to Dr. John E. Howard for permission to study patients under his care and to Dr. Harold Harrison and Dr. Roy Hertz for the blood specimens on their patients.

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Since this report was submitted we have studied two additional patients with Cushing's syndrome due to bilateral adrenal hyperplasia, proven at operation. In both of these patients the hyperresponsiveness to intravenous ACTH previously described was demonstrated. In one instance the plasma level of free corticoids rose from 34 μg/100 ml of plasma to 138 μg at 5 hours. In the second case a control level of 21 μg/100 ml rose to 133 μg at 4 hours and 180 μg at 6 hours.

ABSTRACTS OF PAPERS PRESENTED BEFORE THE JOHNS HOPKINS MEDICAL SOCIETY

December 13, 1954

TOTAL PARENTERAL ALIMENTATION AND THERAPY IN CHRONIC ULCERATIVE COLITIS

MOSES PAULSON

By total parenteral alimentation and therapy is meant feeding solely by non-oral routes, such feeding (including other agents similarly administered) also having therapeutic value.

The sole administration of adequate calories, fluids, electrolytes, carbohydrates and proteins (Amigen) intravenously and vitamins, preferably, intramuscularly was introduced by me at The Johns Hopkins Hospital in May 1945, in chronic indeterminate intestinal inflammatory states. Its use has been restricted principally to certain cases of chronic ulcerative colitis, to a few with ileitis and ileocolitis and in selected cases following re-institution of intestinal continuity after ileostomy for ulcerative colitis.

The method—ordinarily employed from ten to twenty-one days, usually fourteen—and the criteria for the selection of chronic ulcerative colitis cases in which this approach has been found suitable were described. Three-fourths of the group treated have been brought under control within a fortnight, many with an arrest. Re-introduction of food by mouth neither increased defecation nor resulted in reactivation or exaggeration of the disease process.

The therapeutic effectivenss of this approach was not as striking in regional ileitis and ileocolitis, but its place and the nature of the benefits that might be anticipated were pointed out. This method has been helpful too after restoration of intestinal continuity in a few patients with ileostomy for chronic ulcerative colitis.

RENAL EXCRETION PATTERNS OF WATER, SODIUM AND OTHER SUBSTANCES

FRANCIS P. CHINARD AND THEODORE ENNS

Application has been made to the urinary system of the principles used by Stewart and by Hamilton for the estimation of cardiac output by the instantaneous dye injection technique. In the urinary system, injection of the test substances is made into the left renal artery; urine is obtained by means of ureteral catheters from the two kidneys separately. A total of 30 samples or periods, each of 30 seconds duration, is obtained from each kidney. To correct for excretion on recirculation, the amounts of the test substances excreted by the right kidney are subtracted period by period from the corresponding amounts excreted by the left kidney. The amounts (corrected for recirculation) excreted by the left kidney in each sample are divided by the amounts injected to give the corrected weight ratios excreted for each substance. These corrected weight ratios are plotted against time to give the renal excretion patterns. The excretion patterns of glomerular substances (i.e., substances which are assumed to equilibrate across the glomerular capillaries and which are neither reabsorbed, synthesized nor destroyed by the kidney) such as creatinine, inulin, and ferrocyanide, injected in pairs, are nearly identical. The mean transit times (from renal artery to ureteropelvic junction), the extraction ratios (sum of the corrected weight ratios), and the modal transit times are nearly identical for the pairs in each experiment. From the mean transit times and the measured urine flows calculations have been made of a volume assumed to

be the renal dead space (the volume of urine between the glomeruli and the uretero-pelvic junction). Approximate proportionality has been found between urine flow (range 0.5 to 6 ml./min. per kidney) and calculated dead space (range 1.5 to 13 ml.).

Comparison of the renal excretion patterns of p-aminohippurate and glomerular substances shows, as anticipated, that the extraction of PAH is greater than that of the simultaneously injected glomerular substance but in addition that the mean transit time of PAH is significantly greater than that of the glomerular substance. Period by period subtraction of the corrected weight ratios of the glomerular substance from the corrected weight ratios of the PAH is assumed to give the corrected weight ratios for the PAH secreted by the tubules. The mean transit time for secreted PAH is greater than that of the simultaneously injected glomerular substance by approximately 60 seconds. It is assumed that this difference in transit times represents a minimum value for the mean transit time of the conventionally postulated PAH-intracellular complex across the tubular cells. On the assumption that this complex moves across the cell by diffusion, values of the order of five to fifty million are calculated for the particle weight of the complex.

Comparisons have also been made of the renal excretion patterns of glomerular substances and of simultaneously injected carbon 14 labelled urea, of sodium 22, and of labelled water (deuterium or tritium oxides). The pattern for urea is initially lower than but symmetrical to that of the glomerular substances but in the later periods is skewed to the right so that equal or greater fractions of urea are excreted than of the glomerular substance. The hypothesis that urea is reabsorbed by passive back diffusion is compatible with these findings. The crossing of the curves suggests that some of the urea which has entered the tubule cells returns to the tubular lumen. The excretion pattern of water differs markedly from that of glomerular substances: the appearance time is shorter, substantial fractions of labelled water are excreted 30 to 45 seconds before equal fractions of the glomerular substance are excreted, and the mean transit time for water is greater by about 45–60 seconds. In the later periods the weight ratios for water approach the weight ratios for the glomerular substance. The excretion pattern of sodium differs even more markedly from that of glomerular substances: the appearance time, the modal transit time (peak time), and the mean transit time are consistently less by 45 to 60 seconds than the corresponding values for the glomerular substance. Approximately 30 to 45 per cent of the eventually excreted sodium is in the urine before 5 per cent of the simultaneously injected glomerular substance is excreted.

The conventional hypothesis that only sodium not reabsorbed from the tubular lumen is excreted in the urine is not compatible with these experimental results. The simplest explanation is that a substantial fraction of the excreted sodium by-passes a portion or portions of the tubules. This is possible because the mean transit time of blood through the kidney is approximately 6 seconds compared to a mean transit time of urine from glomerulus to uretero-pelvic junction of 2.5 to 3 minutes. Sodium could enter the tubular lumen from the blood and interstitial fluid at the thin segment before a simultaneously injected glomerular substance could reach the same point by way of the proximal tubule.

It is concluded that conventional concepts on the mechanisms of sodium and water excretion and on the functions of the several tubular segments may require revision.

METABOLISM OF INTACT SKELETAL MUSCLE IN MAN: THE ROLE OF CARBOHYDRATE

REUBIN ANDRES, GORDON CADER AND KENNETH L. ZIERLER

Much of our understanding of intermediary metabolism has arisen from studies in which *in vitro* preparations from skeletal muscle have served as a source of enzymes. While such studies yield information unobtainable by other means, they permit conclusions only

about what muscle is capable of doing; they do not provide quantitative assessment of metabolic pathways in the intact organism.

One approach to measurement of the role of various metabolites in intact tissue depends upon the Fick equation which states that the rate at which a metabolite is accepted by a tissue from its environment is the difference between the rate at which the metabolite flows to the tissue and the rate at which it flows from the tissue. Formally stated, $\dot{Q} = F(A - V)$, where $\dot{Q}$ is the rate of uptake of the metabolite by the tissue (and $\dot{Q}$ will have a negative sign if the metabolite is formed in the tissue and released to its environment), F is the fluid flow to and from the tissue, and A and V are, respectively, concentrations of the metabolite in the arterial and venous fluid. By this principle a number of workers have studied certain aspects of the metabolism of brain, liver, kidney and heart in intact man.

Studies in recent years have tended to emphasize the role of carbohydrate as the major substrate oxidized by skeletal muscle. Therefore, initial studies were designed to answer these questions: (1) What is the rate of oxygen consumption in muscle, and (2) What fraction of that oxygen consumption can be accounted for by oxidation of carbohydrate?

The forearm was selected as a convenient muscle mass for this study. Best available estimates indicate that 80 per cent of the metabolically active tissue of the forearm is muscle. Measured were forearm plasma flow and concentrations of interest in brachial artery blood and forearm deep venous blood.

Oxygen and glucose consumption and lactic acid production were measured in ten subjects. In order to answer the second question above, the following assumptions were made:

(1) Glucose taken up from arterial blood may be oxidized to carbon dioxide or metabolized anaerobically to lactic acid which diffuses into venous blood.

(2) Precursors of lactic acid other than glucose (e.g. certain amino acids) are quantitatively of little importance.

(3) Other diffusible intermediates in the glucose metabolic pathway (e.g. pyruvic acid) are also of little quantitative significance.

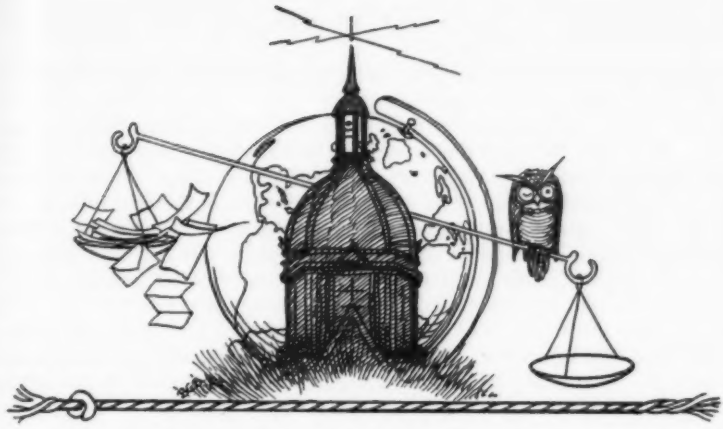
(4) Rate of lactate formation, then, measures that portion of glucose uptake which has been handled anaerobically. The remaining glucose uptake is that portion which has been oxidized.

Oxygen needed to account for all the glucose which has been oxidized can therefore be calculated. If the measured oxygen consumption exceeds this calculated value, then some substrate other than glucose has been oxidized.

The data reveal a high oxygen consumption, 12 μM/min./100 ml. of forearm, compared to a glucose consumption of 1 μM/min./100 ml. Of this total glucose consumption, about one-half was metabolized to lactic acid. Since the oxidation of 1 mole of glucose requires 6 moles of oxygen, an average of only 25 per cent of the oxygen consumption involved glucose. In fact in many of the subjects, virtually no glucose was oxidized.

Theoretically, oxygen not accounted for by glucose uptake might be expended in consumption of glycogen previously stored in muscle. This possibility was tested by measuring the glucose uptake during oral glucose loading. Under these conditions glucose uptake transiently exceeded that needed to account for all the lactic acid produced and oxygen consumed. This excess uptake was probably deposited as glycogen. Calculation of the total glycogen stored revealed, however, that it was inadequate to account for a significant portion of the oxygen consumption during a post-prandial period of many hours.

Neither glucose taken up from arterial blood nor glycogen stored in muscle appears to be the major substrate oxidized in the basal state. If there is a single major substrate, there are hints that it may be lipid, and work in progress is aimed toward quantitation of the role of lipids in metabolism of muscle.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Clinical Orthopaedics, No. 3. Edited by ANTHONY F. DEPALMA. 230 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

This semi-annual publication maintains the good quality of the first two volumes. As in the previous ones the subject matter is divided into two general groups: Section I contains 15 articles on soft tissue conditions around joints, and Section II is made up of 8 articles of miscellaneous orthopaedic interest. Most of the 15 articles in the first part of the book are written very much like chapters in a textbook. Unlike a textbook, however, some of the subject matter in the different chapters cover the same conditions. Inasmuch as each chapter is authored by one or two different experts, there is a variation in the viewpoint concerning similar conditions. This makes very interesting reading for the surgeon of some experience but makes the use of this book by surgeons-in-training confusing or stimulating depending on the student. This is actually no criticism of the editorial policy but shows rather an acceptance by the editor of the fact that few problems in orthopaedics have found a universally accepted solution.

The subjects in the first section include discussions of complete and incomplete ligament tears of joints, lesions of the menisci of the knee, patellar dislocations, tendon avulsions, tenosynovitis of the tendon of the long head of the biceps, and shoulder dislocations. In the second section one finds a thoughtful and thorough review chapter on giant cell tumors of bone; description of a method of knee fusion, and a method of treating Colles' fractures, both by intramedullary fixation; a comparative study of frozen homogenous and autogenous bone grafts; a clear description of the vascular relations of the ankle and their clinical significance. There is also an article which discusses open reduction and primary osteotomy in certain anatomical varieties of femoral neck fractures. There are two case reports:—one of a fatigue fracture of the cortical chip type in the femoral shaft, and another of the use of repository tubocurarine in acute back strain.

ROBERT A. ROBINSON

Biochemical Determinants of Microbial Diseases. By RENÉ J. DUBOS. 152 pp., $3.50. Harvard University Press, Cambridge, Mass.

In 1945, Dr. Dubos published a monograph, *The Bacterial Cell*, which, to quote the author, "dealt with the components, products, and properties of microörganisms which endow them with potential pathogenicity and immunogenic power, and which affect their susceptibility to immunity mechanisms and to chemotherapeutic agents". In the present book, Dr. Dubos summarizes the contributions of the *infected host* to resistance and to susceptibility. Warning in the first chapter that the study of host factors is in a "most primitive state", the author nonetheless summarizes the intricate problems involved in the study of the host. Beginning with a series of examples illustrating how various physiologic alterations can cause "silent infection" to evolve into clinical disease, Dubos then discusses briefly and clearly the differences between the tissues of a living host and the test tube as media for microbial proliferation, the participation of the host in the elaboration and action of various toxins, and some unsolved problems of the host's rôle in tuberculosis. He concludes with a few remarks on controversial issues in immunity and inflammation.

Not only is this an invaluable description of our woefully inadequate factual knowledge in a most important field, it is a brilliant summary of possible methods of approach and new lines of experimental attack upon the intricacies of host participation in the pathogenesis of infection.

I. L. B., JR.

The Autonomic Nervous System, 3rd edition. By JAMES C. WHITE, REGINALD H. SMITHWICK and FIORINDO A. SIMEONE. 569 pp., $12.00. The Macmillan Co., New York, N. Y.

The third edition of this text summarizes the great amount of important data accumulated from wartime and civilian experience during the past decade.

The chapter dealing with the pharmacology of the system is most important and most timely, stressing as it does the chemical mediation of nerve impulses and the drugs and hormones which mimic activity of the autonomic nervous system. In these days when some chemical means is being so actively sought to ablate the parasympathetic nerve activity as in vagotomy for duodenal ulcer, or in ablating the activity of the sympathetic nerves as in the relief of vascular spasm either locally or generally, an understanding of this phase of the subject is indispensable to physicians and surgeons alike.

Excellent presentation is made of the part played by the sympathetic nerves in the various forms of peripheral vascular disease together with the effects which can be expected from sympathectomy. The chapter on the treatment of hypertensive cardiovascular disease is, of course, an authoritative one.

Finally, a complete and detailed description is given of the various operations on the sympathetic nervous system.

I. RIDGEWAY TRIMBLE

Congenital Syphilis. By DAVID NABARRO. 470 pp., $10.00. Edward Arnold, Ltd., London, England. The Williams & Wilkins Co., Baltimore, Md.

To read this book is to savor of old wine, and, like old wine, it is for the connoisseur. Also, like old wine, it is somewhat musty, and contains an inordinate quantity of dregs. It is a book for the syphilologist to read by the firelight of a winter's evening, and cannot be recommended to the busy student or practitioner avid for accurate and practical knowledge.

The author was Medical Officer in charge of Venereal Diseases Clinic at the Hospital for Sick Children, Great Ormond Street, London, from its inception in 1917 until its closure in

1939. The book is largely based upon the nearly 900 cases of congenital syphilis seen during the 23 years of its existence.

The keynote of the volume is set by an excellent illustrated chapter on historical aspects. A mighty march of masters, from Paracelsus to Parran, passes in figured review.

The author exhibits a curiously persistent belief in the nineteenth century theory of paternal transmission and the metaphysics of maternal germ transmission have been disinterred.

The detailed consideration of the symptomatology of congenital syphilis, with numerous illustrative cases from the author's clinic, is, on the whole, excellent. Particularly to be commended are the discussions with photographs and radiographs, of cutaneous manifestations, dental stigmata, and involvement of the bones and joints. A persistent belief in the thoroughly discredited Warthin lesion and in the ubiquity of the *Treponema Pallidum* mar the discussion of cardiovascular involvement in congenital syphilis.

Consideration of central nervous system involvement is fundamentally sound but not entirely adequate. Discussion of the pathological anatomy is sketchy. The clinical picture of juvenile paresis might well have been more vividly painted, and concerning its treatment the author states, "in my view treatment should not be relaxed until blood and C.S.F. are both W.R.-negative and the fluid is normal in all other respects." This is, in most cases, equivalent to a life sentence.

Recommendations as to treatment include a combination of penicillin, mapharsen, and bismuth in repeated courses until the "W.R." becomes negative, and would, in this country at least, be considered archaic.

RICHARD D. HAHN

Therapeutics in Internal Medicine, 2nd edition, completely revised and reset. By Eighty-four Authorities. Edited by FRANKLIN A. KYSER. 830 pp., $15.00. Paul B. Hoeber, Inc., New York, N. Y.

This is the second edition of a rather popular book on therapeutics. It has been completely revised and reset. There are over seventy-five contributors to this volume, but the editor has brought order and system and a fairly uniform style to the diversified writing. Each subject is dealt with by a well recognized authority in the field. Every attempt has been made to keep to the subject—therapy—and pathological anatomy, physiology, and diagnosis are omitted except when a discussion is obviously needed for understanding of the principles expounded.

Any such book dealing with therapy can be recent yet very much out of date. However, this edition incorporates the recent advances in therapeutics and appears to be quite timely. Certainly no major therapeutic advances have been made since this edition was published. The discussions on the use of the adrenal steroids in therapy are complete and timely. Good discussions are presented in the uses and *abuses* of antimicrobial agents. Particularly recommended is the discussion of the therapy of tuberculosis.

Unfortunately the editing of the references and index has not been thorough. There are numerous mistakes in names, volume numbers, and dates. For example, the article so well known in this environment on subacute bacterial endocarditis by Drs. Tumulty and A. M. Harvey is described incorrectly as written by A. P. Tumult and A. H. McGehee.

This volume is recommended as a ready reference for the practitioner for informative and timely discussions on therapy (dosage of drugs, route of administration, effects, toxicity).

The mistakes in the reference detract from the usefulness of the book. It would not be useful in this reviewer's opinion as a text book for medical students or as a reference source for those other than the practitioner.

JOHN COLLINS HARVEY

Annual Review of Medicine, Vol. V. Edited by WINDSOR C. CUTTING. 490 pp., $7.00. Annual Reviews, Inc., Stanford, California.

This review, as with its predecessors, presents analyses of the current literature and thought on various subjects of medical interest. Several of these reviews are excellent in that the author attempts to critically evaluate the literature and to discuss and incorporate seemingly isolated findings. In some instances, however, authors have used this review to merely outline the literature without discussing it, and this eliminates much of its usefulness to the reader. With such a presentation an annotated bibliography would be of as much value. An attempt has been made in some of the reviews to present information of value to the clinician and investigator. This is a hard task and occasionally the practitioner may find much of the review irrelevant to his needs. Considering the review as a whole, however, there is much material of interest and worth study. Two important features of these books are the annotated list of reviews, forming the last chapter, and the extensive bibliography with each topic.

LEIGHTON E. CLUFF

Shock and Circulatory Homeostasis. Transactions of the Third Conference, September 14, 15 and 16, 1953. Edited by HAROLD D. GREEN. 230 pp., $3.50. Josiah Macy, Jr. Foundation, New York, N. Y.

This is a delightful record of discussions held by leaders in the field of circulation and circulatory homeostasis. The recorded informality of the conference makes reading the record a pleasure. Frequent interruptions by the participants do much to clarify ideas and concepts in the reader's mind, as no free use of terms, hedging or equivocation was tolerated by the participants, notably Drs. Heymans, Burton, Dawes and others. The conference was divided into three sections. Dr. John Howard presented "Experiences with shock in the Korean theatre", Dr. G. S. Dawes "Reflex factors in the regulation of the circulation" and Dr. Robert Alexander led a discussion of "The functional properties of blood vessels." On some occasions so many questions were raised concerning gathering or interpretation of data that an orderly train of presentation was lost but usually regained in time by the discussion leader. Particularly in the section led by Dr. Dawes an outline and a complete and orderly discussion is given of the evidence for various reflexes involved in circulatory homeostasis. This is delightfully enlivened by Professor Heymans' questions and comments and is an unusually valuable section.

The informality of the discussion makes the reader feel almost a member of the group. At least this reviewer could visualize his own weariness at conferences and easily understand the situation towards the end when within a few moments, two individuals asked the same simple question and obtained the same simple answer concerning a point which had already been clearly explained.

H. M. BAHNSON

BOOKS RECEIVED FOR REVIEW

Actions of Radiations on Living Cells, 2nd edition. By the late D. E. LEA. 416 pp., $6.00. Cambridge University Press, Cambridge, England.

Angina Pectoris. Entstehung, Erkennung, Beurteilung und Behandlung der Herzschmerzanfälle. By W. H. HAUSS. 294 pp., Ganzleinen DM 59.40 ($14.15). Georg Thieme Verlag, Stuttgart, Germany. Also by Intercontinental Medical Book Corp., New York, N. Y.

Angiopathia Diabetica. Konservative Behandlung des Zuckerbrandes. By M. BÜRGER. 190 pp., Ganzleinen DM 69. ($16.40). Georg Thieme Verlag, Stuttgart, Germany. Also by Intercontinental Book Corp., New York, N. Y.

Cardiac Anomalies. By VINCENT MORAGUES and CHESTER P. LYNXWILER. 92 pp., $6.50. The Williams & Wilkins Co., Baltimore, Md.

Clinical Oethopaedics No. 4. Edited by ANTHONY F. DEPALMA. 240 pp., single numbers $5.00. J. B. Lippincott Co., Philadelphia, Pa.

From Classical to Modern Chemistry. By A. J. BERRY. 251 pp., $4.75. Cambridge University Press, New York, N. Y.

Medical Research Council Special Report Series No. 287. Studies on the Nutritive Value of Bread and on the Effect of Variations in the Extraction Rate of Flour on the Growth of Undernourished Children. By E. M. WIDDOWSON and R. A. MCCANCE. 137 pp., 8s. 6d. net. Her Majesty's Stationery Office, London, England.

Needed Research in Health and Medical Care. A Bio-Social Approach. By CECIL G. SHEPS and EUGENE H. TAYLOR. 216 pp., $5.00. The University of North Carolina Press, Chapel Hill, N. C.

Pediatric Diagnosis. By MORRIS GREEN and JULIUS B. RICHMOND. 436 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

Probleme und Beispiele Biologischer Regulung. By R. WAGNER. 219 pp., Ganzleinen DM 29.40. Georg Thieme Verlag, Stuttgart, Germany. Also by Intercontinental Medical Book Store, New York, N. Y.

Public Relations in Medical Practice. By JAMES E. BRYAN. 301 pp., $5.00. The Williams & Wilkins Co., Baltimore, Md.